ORIGINAL ARTICLE

Pseudo-outbreak of *Mycobacterium abscessus* Infection Caused by Laboratory Contamination

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OBJECTIVE. To investigate the cause(s) of an increased incidence of clinical cultures growing Mycobacterium abscessus at a hospital in Florida.

DESIGN. Outbreak investigation.

SETTING. University-affiliated, tertiary-care hospital.

METHODS. A site visit was done during the first week of September 2006. We reviewed the medical records of patients from whom *M. abscessus* was recovered during the period from January 1, 2003, through June 30, 2006. We collected environmental samples from various sites and evaluated specimen processing procedures in the microbiology laboratory. Isolates of *M. abscessus* recovered from the environment and from 12 randomly selected patients who sought medical care in 2006 were compared by pulsed-field gel electrophoresis (PFGE). Follow-up case surveillance was continued through March 31, 2007.

RESULTS. Specimens from 143 patients obtained from various anatomical sites grew M. abscessus on culture in 2005-2006, compared with specimens from 21 patients in 2003-2004. The 12 isolates from patients that were selected for molecular typing had indistinguishable PFGE patterns. Observations revealed no major breaches in the processing of mycobacterial specimens in the laboratory. Isolates grew only after prolonged incubation (mean \pm SD, 45 \pm 15 days) in test tubes containing diagonally oriented Middlebrook and Cohn 7H10 agar or Lowenstein-Jensen medium. Environmental samples obtained from the inside of the specimen incubator grew M. abscessus on culture. A test tube containing diagonally oriented, uninoculated Middlebrook and Cohn 7H10 agar that was incubated in the same incubator as clinical specimens grew M. abscessus with a PFGE pattern that matched the pattern of the patient isolates. Cases of M. abscessus infection decreased to baseline after the hospital changed suppliers of mycobacterial media and cleaned the incubator.

CONCLUSIONS. Although the source was never confirmed, our investigation suggests that this was a pseudo-outbreak of *M. abscessus* infection that resulted from contamination of mycobacterial cultures during incubation. Our findings emphasize the need for guidance on the disinfection of specimen incubators.

Infect Control Hosp Epidemiol 2008; 29:57-62

Mycobacterium abscessus is a rapidly growing, non-tuberculous, acid-fast bacillus. This species of mycobacterium is ubiquitous in the environment and can be isolated from a variety of sources, including soil, dust, and water. Although M. abscessus is part of the normal flora of the respiratory and digestive tract, it is increasingly being recognized as a human pathogen, particularly of the lungs and of the skin and soft tissue. In the hospital setting, this bacterium can contaminate medical equipment, causing nosocomial infection and outbreaks of infection. A variety of outbreaks have been described in the past that were caused by contaminated surgical instruments, Solutions, and cleaning devices. There

have also been well-documented pseudo-outbreaks that resulted from contaminated laboratory supplies. 13,14

In 2006, a pulmonologist at a Florida hospital recognized that 3 patients who had recently undergone bronchoscopies had cultures of bronchoalveolar lavage fluid samples that were growing *M. abscessus*. An internal evaluation by the infection control department at the hospital demonstrated that the number of cultures growing *M. abscessus* in 2006 was considerably greater than it had been in preceding years. Although a procedure-related outbreak was suspected, environmental sampling of surgical equipment did not reveal a source. Consequently, the hospital requested assistance from

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the county health department and the Centers for Disease Control and Prevention (CDC) to determine the source of the outbreak.

METHODS

We performed case finding through the review of microbiology records. A case patient was defined as a patient who had *M. abscessus* isolated from culture. From these case patient data, an epidemic curve, stratified by the anatomical site from which the culture samples were collected, was created. In this curve, only data for the first culture that grew *M. abscessus* was included for each patient.

To determine whether the recovery of *M. abscessus* represented colonization or infection, we reviewed the medical records of patients who had a culture that grew *M. abscessus* during the period from January 1, 2003, through June 30, 2006. For patients who had culture samples obtained from nonsterile body sites (ie, the maxillary sinus, sputum, or bronchoalveolar lavage fluid), we compared their clinical presentations with the case definitions for sinus, upper respiratory, and pulmonary infections used by the CDC in the National Nosocomial Infections Surveillance (NNIS) system.¹⁵ As another marker of infection, we recorded the results of the acid-fast bacilli stain done at the time the culture sample was obtained. We also assessed whether a patient was treated for *M. abscessus* infection.

We interviewed healthcare staff from the pulmonology, otolaryngology, and infectious diseases services, as well as staff from the surgical processing department and the microbiology laboratory. We also reviewed the protocol by which mycobacterial cultures were processed.

Water samples and surface swab samples (CultureSwab Plus; Becton Dickinson Microbiology Systems) were collected from surgical equipment, such as bronchoscopes and sinus endoscopes, and from several areas of the hospital, including operating rooms, equipment sterilization units, and the microbiology laboratory. Samples were placed on ice and shipped to CDC laboratories on the same day that they were collected. One-liter water samples were transported in bottles containing sodium thiosulfate (0.01% final concentration) to neutralize residual chlorine. An aliquot of each sample was decontaminated with 0.005% cetylpyridinium chloride for 30 minutes at room temperature¹⁶ and processed by membrane filtration. All environmental samples were incubated on plates of R2A agar and plates of Middlebrook 7H10 agar at an ambient temperature or 30°C for 7 days. Isolates were screened with Kinyoun acid-fast staining, followed by highperformance liquid chromatography analysis of mycolic acids for identification.

Twelve isolates of *M. abscessus* recovered from patients on different dates in 2006 and representing a variety of anatomical sites, procedures, and hospital locations were evaluated further by pulsed-field gel electrophoresis (PFGE), in accordance with the protocol for rapidly growing mycobacteria

described elsewhere.^{17,18} The PFGE patterns of these isolates were compared with one another and with the PFGE patterns of isolates from cultures of environmental samples that grew *M. abscessus.*^{18,19}

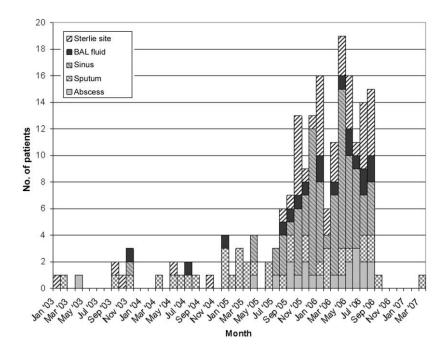
RESULTS

Figure 1 shows the epidemic curve for patients with cultures that grew M. abscessus during the period from January 1, 2003, through March 31, 2007. For each year in this period, the total number of mycobacterial cultures processed by the microbiology laboratory was similar: in 2003, there were 943; in 2004, there were 1,106; in 2005, there were 1,197; and through June of 2006, there were 762. Because the number of patients with cultures that grew M. abscessus increased dramatically in early 2005, we defined the start of the outbreak as January 1, 2005. From January 1, 2005, through June 30, 2006, we identified 143 patients with cultures that grew M. abscessus. Specimens were obtained from various anatomical sites, including the maxillary sinuses (62 samples [43.4%]), abscesses (20 [14.0%]), sputum (20 [14.0%]), bronchoalveolar lavage fluid (11 [7.7%]), and a variety of sterile sites (30 [21.0%]).

During the outbreak period, 50 patients provided samples from a sterile body site and 93 patients provided samples from a nonsterile body site that grew *M. abscessus* on culture. Of the 93 patients with nonsterile body site samples that grew *M. abscessus* on culture, 23 (24.7%) met NNIS criteria for clinical infection. A total of 4 of 143 patients had clinical specimens that were stain positive for acid-fast bacilli. These 4 patients, as well as 4 others, were the only patients to be treated by their clinician for *M. abscessus* infection.

The microbiology laboratory followed standard procedures for processing mycobacterial specimens, and there had been no recent changes in protocol or product suppliers.²⁰ Sterile specimens were inoculated directly onto culture media, unless they were purulent or clotted, in which case they were processed as nonsterile. After processing, mycobacterial cultures were incubated in 3 types of media: test tubes containing diagonally oriented Middlebrook and Cohn 7H10 agar (hereafter, slants) (Becton Dickinson Microbiology Systems), Middlebrook 7H9 broth (Becton Dickinson Microbiology Systems), and Lowenstein-Jensen medium slants (Becton Dickinson Microbiology Systems). These cultures were all placed in a single freestanding CO2 specimen incubator (Forma Scientific) at 35°C, with loosened caps. Our observations revealed no breaches in the processing of mycobacterial specimens, and microbiology laboratory staff reported that no new personnel had prepared mycobacterial specimens for culture during the outbreak period.

According to personnel in the microbiology laboratory, the growth of *M. abscessus* was usually observed on either Middlebrook and Cohn 7H10 agar slants or on Lowenstein-Jensen medium slants. Several lots of each type of medium, which were prepared in advance by the manufacturer, were used in



Number of patients with cultures that grew Mycobacterium abscessus each month from January 2003 through March 2007 at a Florida hospital. The investigation occurred during the first week of September 2006, and the interventions were implemented on September 8, 2006.

the period from January 1, 2005 through June 30, 2006. The same manufacturer supplied both types of media to the hospital. Inquiry to the manufacturer revealed that no lots distributed in 2005-2006 had failed quality assurance checks for mycobacterial contamination. Additionally, the manufacturer had received no other reports from facilities regarding potential media contamination. To further assess the possibility of media contamination, uninoculated Middlebrook and Cohn 7H10 agar slants and Lowenstein-Jensen medium slants were incubated in a specimen incubator at the CDC; these tubes showed no growth.

A review of microbiology laboratory records revealed that the mean $(\pm SD)$ time for growth of clinical isolates of M. abscessus during the outbreak period of 2005-2006 was 45 ± 15 days, which was significantly different (P < .001) from the mean time observed during the preoutbreak period of 2003-2004 (24 \pm 15 days). Furthermore, the fungal contamination rates for mycobacterial cultures processed in the freestanding CO₂specimen incubator were calculated and found to be 5.1% in 2005 and 5.2% in 2006.

On August 3, 2006, uninoculated tubes of each type of medium (ie, Middlebrook and Cohn 7H10 agar, Middlebrook 7H9 broth, and Lowenstein-Jensen medium) were placed in the incubator with loosened caps, to serve as controls. After 28 days of incubation, 1 of the Middlebrook and Cohn 7H10 agar slants grew M. abscessus. Further investigation revealed that the hospital's mycobacterial incubator had been purchased in 1995, was visibly dirty, and had never been cleaned. The incubator contained a water reservoir that was periodically topped off with distilled water, and there was a fan to circulate air. Environmental swab samples, taken from brown, gelatinous material in the gutter at the base of the incubator's glass door were smear-positive for acid-fast bacilli on the day of collection and subsequently grew M. abscessus on culture.

On September 8, 2006, the hospital transferred all of the mycobacterial cultures into a different specimen incubator and changed its supplier of mycobacterial culture media. Soon after, the specimen incubator was thoroughly cleaned in accordance with the manufacturer's instructions. On September 25, 2006, mycobacterial cultures were moved back into the specimen incubator. Figure 1 shows that the rate of cultures that grew M. abscessus returned to baseline after September 2006.

All 12 isolates recovered from patient cultures had identical PFGE patterns; 9 of these are shown in Figure 2, along with the PFGE patterns of isolates recovered from cultures of hospital environmental samples that grew M. abscessus. The PFGE patterns of the isolates recovered from patients matched the PFGE pattern of the isolate obtained from the uninoculated Middlebrook and Cohn agar; however, they were distinct from the PFGE patterns of the M. abscessus isolates collected from the mycobacterial incubator.

DISCUSSION

We describe a large pseudo-outbreak of *M. abscessus* infection involving 143 patients over the course of 18 months. Our findings suggest that the vast majority of these patients were

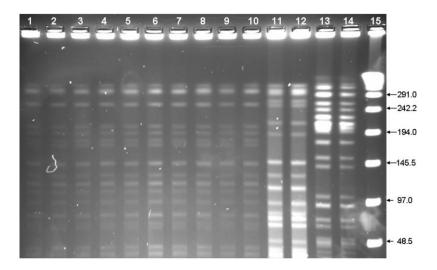


FIGURE 2. Pulsed-field gel electrophoresis patterns of 9 clinical isolates from different patients (*Lanes 1-9*), the isolate recovered from a tube of uninoculated 7H10 Middlebrook agar (*lane 10*), and isolates recovered from culture of environmental samples, including swab samples from the gutter at the base of the specimen incubator's glass door obtained on September 7, 2006 (*lanes 11* and *12*) and September 2, 2006 (*lanes 13* and *14*). *Lane 15*, λ DNA standard. The chromosomal DNA was digested with *XbaI*.

neither colonized nor infected with *M. abscessus*. Although past pseudo-outbreaks of *M. abscessus* caused by laboratory contamination have implicated aqueous solutions, such as a media supplement¹³ and distilled water,¹⁴ we were not able to identify a source in this investigation. Instead, we theorize that the contamination of clinical cultures may have occurred during specimen incubation.

The hypothesis of contamination of mycobacterial cultures is supported by the diversity of specimen types that grew M. abscessus, the lack of clinical findings consistent with infection in case patients, and the infrequency with which clinicians treated case patients for M. abscessus infection. The possibility that the specimen incubator in the microbiology laboratory contributed to this contamination is strengthened by the observations that the incubator had never been cleaned and that it had levels of fungal contamination above the acceptable range of 3%-5%.²¹ In addition, there are only a limited number of ways to explain how an uninoculated slant of Middlebrook and Cohn 7H10 media could grow the pseudooutbreak strain of M. abscessus. These possibilities include contamination of the slant when the mycobacterial cultures were checked for growth each week by microbiology laboratory personnel, sabotage, contamination of the media during preparation by the manufacturer, or contamination by M. abscessus circulating in the specimen incubator.

Our findings argue against all but one of these possibilities. It is unlikely that contamination of the slants was occurring when cultures were observed for growth because the slant caps are not removed as part of this protocol. There was also no clear motive for sabotage. Although contamination of media with a non–tuberculous mycobacterium at the time of production has been observed on at least 2 occasions in the past, ^{22,23} in this instance there is no evidence to support this

hypothesis either. Over the course of the 18-month outbreak period, the microbiology laboratory used several lots of media. These media was obtained directly from the manufacturer, and no supplements were added by the microbiology laboratory before inoculation. According to the microbiologist at this hospital, *M. abscessus* was observed growing in both Lowenstein-Jensen slants and Middlebrook and Cohn 7H10 slants. Such prolonged and widespread contamination of nationally distributed media would have been recognized during quality assurance checks by the manufacturer and in other facilities.

The most likely explanation of the pseudo-outbreak is that the cultures were contaminated by a strain of M. abscessus circulating in the specimen incubator. Several findings lend support to this theory. First, environmental sampling demonstrated that at least 2 strains of M. abscessus were present in the incubator, suggesting that several other strains of M. abscessus, including the pseudo-outbreak strain, may have been present. Second, an uninoculated tube of medium placed in the incubator grew M. abscessus after 28 days of incubation. Third, the prolonged time for observed growth is consistent with contamination during, rather than prior to, incubation. Although temperature and inoculum size may affect growth rate,24 rapidly growing mycobacteria tend, in general, to grow in culture within 7 days1; this strain grew that quickly in pure culture at the state laboratory. Finally, this possibility is supported most directly by the fact that the pseudo-outbreak ended after the incubator was cleaned. Though the facility did change media suppliers as well, our findings indicate it was the cleaning of the incubator, and not the change in media supply, that led to the termination of this outbreak.

The major limitation of this investigation is that environ-

mental sampling did not identify the same strain of M. abscessus in the environment as was seen in patient samples. Consequently, we may never know the specific cause of this pseudo-outbreak. Although 2 different strains were recovered from the incubator, these strains did not match the clonal isolate found in all 12 patient samples and in the uninoculated media. It is possible that PFGE of all of the colonies growing in cultures of environmental samples would have detected the outbreak strain. The clonal nature of the outbreak may have resulted from the fact that, among the environmental strains of M. abscessus, the outbreak strain was better adapted for growth on media.

During the course of the investigation, it became apparent that the microbiology laboratory at this hospital lacked a policy for routine cleaning of its mycobacterial specimen incubator. The internal environment of incubators is obviously designed to promote the growth of microorganisms. The presence of water pans and condensation in specimen incubators create the potential that the interior surfaces will become colonized with organisms, such as rapidly growing mycobacteria. The presence of a fan to circulate air likewise creates a mechanism by which environmental contaminants might circulate in the incubator. During this pseudo-outbreak, the contamination of cultures may have occurred through the aerosolization of water colonized with M. abscessus, which then entered culture tubes through the loosened caps of the slants used for mycobacterial culture. Because the loosening of caps is required to allow CO2 to enter, the best way to prevent this kind of contamination is by periodic cleaning of the incubator. That way, only isolates from samples will grow on culture, rather than contaminants.

Although the manufacturer's instructions for this specimen incubator recommend "periodic" cleaning, they did not specifically recommend a cleaning schedule. The 1992 edition of the American Society for Microbiology Clinical Microbiology Procedures Handbook includes a section on standard incubator maintenance and recommends that specimen incubators be cleaned "inside and out" at least once a year and "as needed" (after spills, for example). 25(p12.12.1-12.12.8) The 2004 version of this handbook, however, does not include the section outlining incubator maintenance.26 Conversations with the editors of this handbook have indicated that there were no problems with the previous recommendations and that these recommendations could still be followed. Therefore, clinical microbiologists should review the cleaning protocols for specimen incubators that are recommended both by manufacturers and the American Society for Microbiology and should implement protocols to ensure that these recommendations are followed. In addition, this pseudo-outbreak should serve as a reminder to microbiology laboratories and infection control personnel to monitor the incidence rates of cultures positive for non-tuberculosis causing mycobacteria, as well as other pathogens, so that potential problems like this one can be addressed as early as possible.

ACKNOWLEDGMENTS

We thank Michael J. Miller and Michael Bell for their review of the manuscript.

Financial support. The investigation was funded through the Office of Workforce and Career Development, Centers for Disease Control and Prevention, Atlanta, Georgia.

Potential conflicts of interest. All authors report no conflicts of interest relevant to this article.

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Presented in part: 17th Annual Scientific Meeting of the Society for Healthcare Epidemiology of America; Baltimore, Maryland; April 2007 (Abstract

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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